

cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, in an excessive amount over a level dissolving completely at respective temperatures, cap-sealed, and stirred for two days while keeping at respective temperatures of 10-90°C until being saturated. ~~The—Each~~ resulting ~~each~~-saturated solution of cyclotetrasaccharide was membrane filtered to remove undissolved cyclotetrasaccharide, and each filtrate was then examined for moisture content by the drying loss method to determine a saturation concentration of cyclotetrasaccharide at respective temperatures. The results are in Table 34.

ah. at page 159, paragraph 1 <sup>3</sup>

A crystalline cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, and a commercialized polypeptone, Nihonseiyaku K.K., Tokyo, Japan, were dissolved in deionized water to obtain a 10% (w/v) cyclotetrasaccharide solution containing 5% (w/v) polypeptone. Four milliliters of the resulting solution were placed in a glass test tube, sealed, and heated at 100°C for 30 to 90 min. After allowing to stand for cooling at ambient temperature, each of the resulting ~~solution~~-solutions was measured for coloration degree to examine on their amino carbonyl reactivity. In parallel, as a control, a solution with only polypeptone was provided and similarly treated as above. The coloration degree was evaluated based on the level of the absorbance, measured in a cell with 1-cm light pass at a wavelength of 480 nm, minus the ~~one of the control~~. The results are in Table 38.

reaction, the reaction mixture was heated at 100°C for 15 min to inactivate the remaining enzymes. Then, the reaction mixture thus obtained was treated with  $\alpha$ -glucosidase and glucoamylase similarly as in Experiment 1 to hydrolyze the remaining reducing oligosaccharides, followed by quantifying the formed cyclotetrasaccharide on HPLC. The results are in ~~Fale~~Table 31.

ac. at page 148, paragraph 2

As is evident from ~~he~~the results in Table 32, the formation yield of cyclotetrasaccharide was about 64% at a low concentration of 0.5%, while it was about 40% at a high concentration of 40%. The fact indicated that the formation yield of cyclotetrasaccharide increased depending on the concentration of partial starch hydrolyzate as a substrate. The result revealed that the formation yield of cyclotetrasaccharide increased as the decrease of partial starch hydrolyzate.

ad. <sup>150</sup>at page 151, paragraph 1 <sup>2</sup>

The saccharide solution was fed to a column packed with about 225 L of "AMBERLITE CR-1310 (Na-form)", an ion-exchange resin commercialized by Japan Organo Co., Ltd., Tokyo, Japan, and chromatographed at a column temperature of 60\_C and a flow rate of about 45 L/h. While the saccharide composition of eluate from the column was ~~monitoring~~monitored by HPLC as described in Experiment 1, fractions of cyclotetrasaccharide with a purity of at least 98% were collected, and in a usual manner desalted, decolored, filtered, and concentrated to obtain about 7.5 kg of a saccharide solution with a solid content of about 2,500 g solids. HPLC measurement for saccharide composition of the

w. at page 133, paragraph 2

To study whether the  $\alpha$ -isomaltosylglucosaccharide-formation enzyme of the present invention has the ability of forming to form dextran, it was tested in accordance with the method in *Bioscience Biotechnology and Biochemistry*, Vol. 56, pp. 169-173...

x. at page<sup>133a</sup> 134

To study whether the  $\alpha$ -isomaltosylglucosaccharide-formation enzyme of the present invention has the ability of forming dextran, it was tested in accordance with the method in *Bioscience Biotechnology and Biochemistry*, Vol. 56, pp. 169-173 (1992). To a 1% (w/v) aqueous solution of maltotetraose as a substrate was added 0.25 unit/g substrate, d.s.b., of either of purified specimens of  $\alpha$ -isomaltosylglucosaccharide-forming enzyme from *Bacillus globisporus* C9 obtained by the method in Experiment 4-2, *Bacillus globisporus* C11 obtained by the method in Experiment 7-2, *Bacillus globisporus* N75 obtained by the method in Experiment 11-2, or *Arthrobacter globiformis* A19 obtained by the method in Experiment 15-2, and incubated at 35°C and pH 6.0, except that pH 8.4 was used for the enzyme from *Arthrobacter globiformis* A19, for four or eight hours. After completion of the enzymatic reaction, the reaction was suspended by heating at 100°C for 15 min.. Fifty microliters of each of the reaction mixtures were placed in a centrifugation tube and then admixed and sufficiently stirred with 3-fold volumes of ethanol, followed by standing at 4°C for 30 min. Thereafter, each mixture solution was centrifuged at 15,000 rpm for five minutes and, after removing supernatant, the resulting sediment was admixed with one milliliter of 75% (w/w) ethanol solution and stirred for washing. The-Each resulting each-solution was

cake mix, instant juice or soft drink, instant coffee, "sokuseki-shiruko" (an instant mix of adzuki-bean soup with rice cake), and instant soup mix; and other foods and beverages such as solid foods for babies, foods for therapy, health/tonic drinks, peptide foods, and frozen foods. The cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrarily used to prolong or retain the flavor and taste of fresh-baked Japanese and Western confectioneries and to improve the taste preference of feeds and pet foods for animals and pets such as domestic animals, poultry, honey bees, silk ~~worms~~silkworms, and ~~fishes~~fish; and also they can be arbitrary arbitrarily used as a sweetener, taste-improving agent, flavoring substance, quality-improving agent, and stabilizer in other products in a paste or liquid form such as a tobacco, cigarette, tooth paste, lipstick, rouge, lip cream, internal liquid medicine, tablet, troche, cod liver oil in the form of drop, cachou, oral refrigerant, gargle, cosmetic, and pharmaceutical. When used as a quality-improving agent or stabilizer, the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrarily used in biologically active substances susceptible to lose their effective ingredients and activities, as well as in health foods and pharmaceuticals containing the biologically active substances. Examples of such biologically active substances are liquid preparations containing lymphokines such as  $\alpha$ -,  $\beta$ - and  $\gamma$ -interferons, tumor necrosis factor- $\alpha$  (TNF-...

d. at page <sup>45</sup> ~~48~~

$\alpha$ ), tumor necrosis factor- $\beta$  (TNF- $\beta$ ), macrophage migration inhibitory factor, colony-stimulating factor, transfer factor, and interleukin 2; liquid preparations containing hormones such as insulin, growth hormone, prolactin, erythropoietin, and